

Diagnostic and Prognostic Biomarkers for Chronic Fibrosing Interstitial Lung Diseases With a Progressive Phenotype



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Biomarkers have the potential to become central to the clinical evaluation and monitoring of patients with chronic fibrosing interstitial lung diseases (ILDs) with a progressive phenotype. Here we summarize the current understanding of putative serum, BAL fluid, and genetic biomarkers in this setting, according to their hypothesized pathobiologic mechanisms: evidence of epithelial cell dysfunction (eg, Krebs von den Lungen-6 antigen), fibroblast proliferation and extracellular matrix production or turnover (eg, matrix metalloproteinase-1), or immune dysregulation (eg, CC chemokine ligand 18). While most of the available data come from idiopathic pulmonary fibrosis (IPF), the prototypic progressive fibrosing ILD, data are available in the broader patient population of chronic fibrosing ILDs. A number of these biomarkers show promise, however, none have been validated. In this review article, we assess both the status of proposed biomarkers for chronic fibrosing lung diseases with a progressive phenotype in predicting disease risk or predisposition, diagnosis, prognosis, and treatment response and provide a direct comparison between IPF and other chronic fibrotic ILDs. We also reflect on the current clinical usefulness and future direction of research for biomarkers in the setting of chronic fibrosing ILDs with a progressive phenotype.

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ABBREVIATIONS: BALF = BAL fluid; CA = cancer antigen; CC16 = 16-kDa Clara cell secretory protein; CCL = C-C motif chemokine ligand; CRP = C-reactive protein; CTD-ILD = connective tissue disease-associated ILD; CXCL = C-X-C motif chemokine; DLCO = diffusing capacity of the lungs for carbon monoxide; ECM = extracellular matrix; HLA = human leukocyte antigen; HP = hypersensitivity pneumonitis; IGF = insulinlike growth factor; IGFBP = IGF-binding protein; ILD = interstitial lung disease; iNSIP = idiopathic nonspecific interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; KL-6 = Krebs von den Lungen-6; LOXL2 = lysyl oxidase-like 2; MMP = matrix metalloproteinase; MX1 = myxovirus resistance protein 1; RA = rheumatoid arthritis; RA-ILD = RA-associated ILD; S100 = S100 calcium-binding protein; SNP = single nucleotide polymorphism; SP-A = surfactant protein A; SP-D = surfactant protein D; SSc = systemic sclerosis; SSc-ILD = SSc-associated ILD; VEGF = vascular endothelial growth factor; YKL-40 = chitinase-3-like protein 1

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Over 200 distinct pulmonary disorders are under the heading of interstitial lung disease (ILD), with idiopathic pulmonary fibrosis (IPF) being the most recognized.¹ Characterized by the development of progressive pulmonary fibrosis, IPF results in lung function and quality-of-life deterioration and worsening respiratory symptoms.² A similar chronic progressive fibrosing phenotype occurs in varying proportions of patients with other fibrotic ILDs—for example, idiopathic nonspecific interstitial pneumonia (iNSIP), hypersensitivity pneumonitis (HP), systemic sclerosis (SSc)-associated ILD (SSc-ILD), rheumatoid arthritis (RA)-associated ILD (RA-ILD), and sarcoidosis. Although no formal definition of “progressive” exists, Cottin et al³ suggest that patients meeting any of the following criteria within a 24-month period have experienced disease progression: a relative decline of $\geq 10\%$ in FVC, a relative decline of $\geq 15\%$ in diffusing capacity of the lungs for carbon monoxide (DLCO), or worsening symptoms or radiologic appearance accompanied by a $\geq 5\%$ relative decrease in FVC. All patients with ILD with a chronic progressive fibrosing phenotype share some clinical, radiologic, and pathologic characteristics.^{4–8} The prognosis is broadly consistent across cohorts of individuals with chronic fibrosing ILDs with a progressive phenotype, strengthening the rationale for grouping these diseases. However, determining an individual patient’s risk of progression, long-term prognosis, and likelihood of treatment response is challenging because of the intrinsic variability seen among patients with the same diagnosis.

A biomarker may be defined as “any substance, structure, or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease.”⁹ For the purposes of this article, we have considered molecular (protein and RNA) markers that can be quantified in biological tissue or fluids (eg, whole blood, serum, BAL fluid [BALF], induced sputum) that reflect physiologic or pathologic processes or that reflect pharmacologic responses to a therapeutic intervention.¹⁰ Nonprotein biomarkers (eg, mitochondrial markers, microRNA, quantitative imaging, cell counts in BALF, lung microbiome analyses, lung physiology) are beyond the scope of this article. Biomarker development has been identified as a key step toward establishing personalized medicine.¹¹ In the field of chronic fibrosing ILDs with a progressive phenotype, biomarker development aims to establish easily measurable variables that allow improved clinical

classification of different ILDs, predict prognosis or likelihood of response to therapy, or monitor treatment response.¹²

This review describes the typical classification of currently proposed biomarkers according to their hypothesized pathobiologic mechanisms: alveolar epithelial cell injury, inflammation and fibrosis, tissue remodeling and repair, and immunologic changes.^{10,13} We subsequently examine the most promising serum and BALF biomarkers and propose to classify them by whether they are associated with disease predisposition, diagnosis, disease progression and prognosis, or response to treatment. We elaborate this classification further by directly comparing biomarker profiles of IPF and non-IPF chronic fibrosing ILDs with a progressive phenotype. Finally, we provide an analysis of the current clinical usefulness of biomarkers in fibrosing ILDs and offer recommendations for future biomarker development and research directions.

Candidate Biomarkers by Mechanistic Pathway

Some of the most promising biomarkers of chronic fibrosing ILDs with a progressive phenotype are markers of proposed mechanisms involved in disease pathogenesis.

Epithelial Cell Dysfunction

A number of molecules are markers of alveolar epithelial cell injury or regeneration.¹⁴ Krebs von den Lungen-6 (KL-6), a submolecule of mucin 1,¹⁵ is a glycoprotein expressed in type II pneumocytes and bronchiolar epithelial cells that may be involved in promoting the migration, proliferation, and survival of lung fibroblasts.¹⁶ The mucin gene *MUC5B* encodes mucin 5B, a major gel-forming protein in human airway secretions that has been linked to the maintenance of airway health.¹⁷ Surfactant protein A (SP-A) and surfactant protein D (SP-D) are lipoprotein complexes secreted by type II pneumocytes and airway cells; they are involved in stabilizing alveolar surface tension at the air-liquid interface and supporting lung host innate immunity.¹³ Chitinase-3-like protein 1 (YKL-40) is a chitinase-like protein involved in innate immune system and cell processes in relation to extracellular matrix (ECM) remodeling.¹³

ECM Turnover

The development of pulmonary fibrosis is characterized by increased turnover of ECM. Transforming growth factor- β plays an important role but has not been widely

explored as a pulmonary fibrosis biomarker due to its ubiquity and the difficulty of accurately quantifying it. Other markers of ECM production and turnover, however, appear more promising. Matrix metalloproteinases (MMPs) are zinc-dependent protease enzymes regulating ECM remodeling.^{13,18} Lysyl oxidase-like 2 (LOXL2) catalyzes the cross-linking of collagen and has been identified as a key mediator of fibrosis.¹⁹ It is highly expressed in fibrosing lungs and is believed to play key roles in ECM remodeling and fibrogenesis.^{10,13,20} Insulinlike growth factor (IGF) has been shown to stimulate the production of ECM by fibroblasts and to encourage epithelial cells to proliferate. IGF-binding proteins (IGFBPs) contribute to IGF activity by facilitating transportation and receptor binding of IGF.^{21,22} Vascular endothelial growth factor (VEGF) is a growth factor with proangiogenic activity and is believed to help protect the epithelium from injury and to encourage tissue repair.²³ In response to lung injury, airway club cells produce and release a low-molecular-weight protein, the 16-kDa Clara cell secretory protein (CC16).¹³ Periostin is an ECM protein which belongs to the fasciclin family; serum levels of periostin increase in IPF and other fibrotic idiopathic interstitial pneumonias and are associated with declines in DLCO and vital capacity.²⁴

Immune Dysregulation

These biomarkers include chemokines, cytokines, and their receptors, of which the most promising are C-C motif chemokine ligand (CCL) 18 and IL-6.¹³ CCL18 is derived from alveolar macrophages and appears to have numerous functions beyond its role as a chemoattractant, including the regulation of fibrosis.²⁵ Levels of the proinflammatory and profibrotic cytokine

IL-6 are increased in serum and BALF in many pulmonary diseases.¹³

Biomarker Applications in Chronic Fibrosing ILDs With a Progressive Phenotype

Risk and Predisposition Biomarkers

Data in IPF: Some genetic variants have been associated with an increased likelihood of developing IPF (Table 1).²⁶⁻³⁹ Studies suggest that the single nucleotide polymorphism (SNP) rs35705950 in the promoter region of the *MUC5B* gene increases the risk of developing IPF, although it is associated with a less progressive form of the disease.²⁶ *TOLLIP* encodes the toll-interacting protein, which regulates immune responses mediated through toll-like receptors, including the modulation of transforming growth factor- β signaling.⁴⁰ Three SNPs in the *TOLLIP* gene have been linked with susceptibility to IPF.³² Telomerase complex genes include *TERT* and *TERC*, which encode telomerase reverse transcriptase and RNA component, respectively. Heterozygous mutations in either the *TERT* or *TERC* genes, or shortened telomeres, may be associated with an increased risk of IPF.²⁹ In addition to these variants in *MUC5B*, *TOLLIP*, or *TERT* and *TERC*, a recent resequencing study of 3,624 IPF and 4,442 control samples highlighted that rare variants in the *FAM13A* and *RTEL1* genes were also contributing to the genetic risk of developing IPF.³¹ Human leukocyte antigen (HLA) class I and II histocompatibility genes encode HLAs, which regulate the immune response to presenting antigens. The HLA-A*02-DRB1*04 haplotype has been associated with genetic susceptibility for IPF.³³

TABLE 1] Risk and Predisposition Biomarkers

Disease	Mechanistic Pathway	Biomarker	Disease Subcategory ^a
IPF	Epithelial cell dysfunction and ECM remodeling	<i>MUC5B</i> ²⁶⁻²⁸ <i>TERT</i> , <i>TERC</i> ^{29,30} <i>FAM13A</i> , <i>RTEL1</i> ³¹	...
	Immune dysregulation	<i>TOLLIP</i> ³² HLA ³³	...
Chronic fibrosing ILDs with a progressive phenotype	Epithelial cell dysfunction and ECM remodeling	<i>MUC5B</i>	RA-ILD ³⁴
	Immune dysregulation	HLA	Sarcoidosis, ^{35,36} SSc-ILD, ³⁷ RA-ILD ^{38,39}

HLA = human leukocyte antigen; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; RA-ILD = rheumatoid arthritis-associated ILD; SSc-ILD = systemic sclerosis-associated ILD.

^aFor chronic fibrosing ILDs with a progressive phenotype.

TABLE 2] Diagnostic Biomarkers

Disease	Mechanistic Pathway	Biomarker	Disease Subcategory ^a
IPF	Epithelial cell dysfunction and ECM remodeling	MMP-1, MMP-7, and others MMPs ⁴³⁻⁴⁵ IGFBP-2 ^{46,47} VEGF ^{23,48,49} Periostin ²⁴ PAI-1 ⁵⁰	...
	Immune dysregulation	CXCL13 ²⁷ S100A8, S100A9 ^{13,51}	...
Chronic fibrosing ILDs with a progressive phenotype	Epithelial cell dysfunction and ECM remodeling	KL-6 SP-A, SP-D CC16 MMP-1, MMP-7, MMP-12 TIMP-1 Periostin	IIP, HP, CTD-ILD, sarcoidosis, asbestosis, iNSIP ^{13,38,52-55} HP, iNSIP, SSc-ILD ^{13,38,56,57} SSc-ILD, sarcoidosis, asbestosis ⁵⁸⁻⁶⁰ Sarcoidosis, RA-ILD, SSc-ILD ⁶¹⁻⁶⁷ SSc-ILD ^{13,38} NSIP, cryptogenic organizing pneumonia ²⁴
	Immune dysregulation	CCL18, CCL2 CCL15, CCL18 S100A8, S100A9 CXCL10 IL-4, IL-6, IL-7, IL-8 IL-12, IL-18, sIL-2R Anti-topoisomerase I, anti-U1 RNP, anti-U3 RNP, anti-U11/U12 RNP, anti-endothelial cell antibodies CRP SAA Anti-MX1	SSc-ILD ⁶⁸⁻⁷² Sarcoidosis ^{42,73} iNSIP, ⁷⁴ SSc-ILD ^{13,75} RA-ILD, ⁶⁴ sarcoidosis ⁴² SSc-ILD ^{63,69,76} Sarcoidosis ^{42,77} SSc-ILD ³⁸ Sarcoidosis ^{42,77} Sarcoidosis ^{42,77} iNSIP ⁷⁸

CC16 = 16-kDa Clara cell secretory protein; CCL = C-C motif chemokine ligand; CRP = C-reactive protein; CTD-ILD = connective tissue disease-associated ILD; CXCL = C-X-C motif chemokine; HP = hypersensitivity pneumonitis; IGFBP = insulinlike growth factor-binding protein; IIP = idiopathic interstitial pneumonias; iNSIP = idiopathic nonspecific interstitial pneumonia; KL-6 = Krebs von den Lungen-6; MMP = matrix metalloproteinase; MX1 = myxovirus resistance protein 1; PAI-1 = plasminogen activator inhibitor-1; RNP = ribonucleoprotein; S100 = S100 calcium-binding protein; SAA = serum amyloid A; sIL-2R = soluble IL-2 receptor; SP-A = surfactant protein A; SP-D = surfactant protein D; TIMP-1 = tissue inhibitor of metalloproteinase-1; VEGF = vascular endothelial growth factor. See Table 1 legend for expansion of other abbreviations.

^aFor chronic fibrosing ILDs with a progressive phenotype.

Data in Other Chronic Fibrosing ILDs With a Progressive Phenotype:

As described earlier, there is evidence that individuals with the SNP rs35705950 in the *MUC5B* gene have an increased risk of developing IPF; this SNP is also associated with an increased risk of RA-ILD.³⁴ However, rs35705950 does not appear to be associated with pulmonary fibrosis in SSc, sarcoidosis, or myositis-associated ILD.⁴¹

Certain HLA haplotypes are also associated with the development of non-IPF ILDs. The HLA-DRB1*1501/HLA-DQB1*0602 haplotype, for example, has been associated with chronic sarcoidosis.⁴² The rare *HLADRB5*01:05* allele may predict the development of ILD in patients with SSc.³⁷ There is also evidence that specific HLA alleles, including *HLA-DRB1*1502*, are

associated with an increased risk of ILD in patients with RA.^{38,39}

Diagnostic Biomarkers

Data in IPF: Elevations in levels of MMP-1 (serum), MMP-7 (serum, BALF, and induced sputum), and other MMPs are recognized in IPF (Table 2).^{13,43-78} In addition, serum and sputum from patients with IPF display significantly higher levels of IGFBP-2 ($P < .001$) compared with those from healthy subjects.^{46,47}

Increased circulating levels of C-X-C motif chemokine (CXCL) 13 have been observed in patients with IPF vs that in healthy control subjects.²⁷ Elevated levels of the proinflammatory monocyte/macrophage-derived calcium-binding proteins, S100 calcium-binding protein

(S100)A8 (also known as calgranulin A—levels increased in plasma) and S100A9 (calgranulin B—levels increased in BALF), have been found in IPF.^{13,51}

Although the numbers of circulating fibrocytes (precursors of fibroblasts) have been suggested to be increased in patients with IPF,^{13,79} there are contradictory data regarding VEGF and its role. Serum levels of VEGF have been shown to be elevated in patients with IPF,⁴⁸ whereas BALF levels appear to be reduced in patients with IPF vs that in healthy control subjects.^{23,49}

Data in Other Chronic Fibrosing ILDs With a Progressive Phenotype: Elevated KL-6 concentrations have been reported in the serum and BALF of patients with idiopathic interstitial pneumonia, HP, pulmonary sarcoidosis, asbestosis, and connective tissue disease-associated ILDs (CTD-ILDs).^{13,38,52-55,80} Recently, BALF levels of both KL-6 and S100A9 were also found to be significantly higher in patients with fibrotic iNSIP than in healthy subjects and were similar to those in patients with IPF.⁷⁴ Serum levels of SP-A and/or SP-D are increased in fibrosing ILDs (eg, HP, iNSIP, SSc-ILD); high levels of these lipoproteins have been associated with a progressive phenotype in patients with iNSIP and with decreases in DLCO and FVC.^{13,38,56} Increased concentrations of the chemokine CC16 have also been observed in the serum and BALF of patients with pulmonary sarcoidosis, asbestosis, and SSc-ILD.⁵⁸⁻⁶⁰

Increased levels of MMP-1 and MMP-7 have been reported in sarcoidosis, RA-ILD, and SSc-ILD.⁶¹⁻⁶³ Serum levels of MMP-7 are significantly higher in patients with RA-ILD vs those in patients with RA without ILD.⁶⁴ Increased serum and/or BALF levels of other MMPs and tissue inhibitors of MMPs (tissue inhibitor of metalloproteinases) have been reported in various ILDs.^{13,38} For example, BALF levels of MMP-2 are higher in patients with nonspecific interstitial pneumonia than in those with IPF.⁶⁵ It was reported that, among patients with elevated MMP-7, the absence of concurrent increases in SP-D and osteopontin suggests the presence of a non-IPF or unusual interstitial pneumonia pattern ILD.⁶⁶ Patients with sarcoidosis have increased expression of MMP-12 vs control subjects, with correlation between expression levels and disease severity.⁶⁷ An increased number of circulating fibrocytes has been observed in patients with RA-ILDs.⁸¹ Just as for IPF, serum levels of S100A8 and S100A9 were reported to be elevated in SSc, with the highest levels in patients with lung fibrosis.^{13,75}

A number of chemokines have shown correlations with the presence of ILD. Elevated levels of CCL18 have been

found in the serum, BALF, and lung tissue of patients with SSc-ILD and other chronic fibrosing ILDs with a progressive phenotype.⁶⁸ In sarcoidosis, levels of CCL18 appear to correlate with the extent of disease activity.⁷³ Increased BALF and serum levels of CCL2 were seen in patients with SSc and have been shown to correlate with the presence of ILD.⁶⁹ High levels of CCL15 have been associated with progressive sarcoidosis.⁴² CXCL10 and CXCL11 have also been identified as possible diagnostic markers for this disease, with the possibility of CXCL10 enabling differentiation between active and inactive forms.⁴² Patients with RA with ILD have significantly higher serum CXCL10 levels than do those without ILD.⁶⁴ The cytokine IL-6, known to be elevated in a variety of inflammatory diseases (such as sepsis),⁷⁶ was found to be increased in the BALF of patients with SSc-ILD, alongside IL-4, IL-7, and IL-8.^{63,69} Increased levels of interleukins (eg, IL-12, IL-18) have been associated with sarcoidosis.^{42,77} Several other biomarkers related to immune function—soluble IL-2 receptor (sIL-2R), C-reactive protein (CRP), and serum amyloid A (serum amyloid A)—have shown high sensitivity for sarcoidosis.⁷⁷

Serum autoantibodies and immunologic proteins associated with pulmonary fibrosis in SSc-ILD include anti-topoisomerase I antibodies, autoantibodies to small nuclear ribonucleoproteins (RNPs; eg, anti-U1, -U3 and -U11/U12 RNPs), and anti-endothelial cell antibodies.³⁸ Serum autoantibodies against myxovirus resistance protein 1 (MX1) have been identified as a possible diagnostic biomarker for iNSIP.⁷⁸ Other potential serum and BALF biomarkers that have been investigated include circulating cells and molecules associated with macrophage or monocyte activation or endothelial cell injury.^{13,38,82}

Chitotriosidase is an enzyme produced by alveolar macrophages in patients with sarcoidosis. Detected in BALF, chitotriosidase levels have been reported to correlate with the severity of sarcoidosis.⁴²

Prognostic Biomarkers

Data in IPF: Threshold serum levels of KL-6, or sequential changes in KL-6 levels, have been shown to predict lung function decline or outcome in patients with IPF (Table 3).⁸³⁻¹³¹ In addition, increased serum and BALF YKL-40 levels have been shown to predict lower survival rates,⁹¹ and high levels of circulating fibrocytes have been associated with increased risk of mortality.^{13,79} In patients with IPF, elevated MMP-7 was strongly associated with reduced survival.^{44,86,87} High serum levels of SP-A and/or SP-D have been associated

TABLE 3] Prognostic Biomarkers

Disease	Mechanistic Pathway	Biomarker	Disease Subcategory ^a
IPF	Epithelial cell dysfunction and ECM remodeling	KL-6 ⁸³⁻⁸⁵ MMP-7 ^{47,86,87} SP-A, SP-D ^{86,88-90} YKL-40 ⁹¹ ICAM-1, VCAM-1 ⁸⁷ MUCB5, TOLLIP ^{92,93} TERT, TERC ^{94,95} CA 19-9 ⁹⁶ CA-125 ⁹⁶ Tenascin C ⁹⁷	...
	Immune dysregulation	CCL18 ^{89,98,99} IL-6, IL-8 ^{87,100} LOXL2 ^{19,27} S100A12 ⁶¹	...
Chronic fibrosing ILDs with a progressive phenotype	Epithelial cell dysfunction and ECM remodeling	KL-6 SP-A, SP-D YKL-40 MMP-7 MMP-12, TIMP-1 CC16 Tenascin C CA 19-9 CA-125 VCAM-1	iNSIP, HP, CTD-ILD, SSc-ILD ^{56,57,74,101-112} iNSIP, HP, SSc-ILD ^{13,38,57,113} HP, SSc-ILD, sarcoidosis ¹¹⁴⁻¹¹⁷ HP ¹¹⁸ SSc-ILD ^{119,120} SSc-ILD ⁶⁰ SSc-ILD, sarcoidosis, HP ^{97,121,122} CTD-ILD, SSc-ILD ¹²³⁻¹²⁵ CTD-ILD, SSc-ILD ^{118,123-125} CTD-ILD, HP ¹¹⁸
	Immune dysregulation	S100A9 CCL2, CCL18 IL-6, IL-2 CRP IFN-γ CXCL4, CXCL10, CX3CL1 CXCL13 Anti-MX1 Anti-citrullinated protein Chitotriosidase	iNSIP ⁷⁴ SSc-ILD ^{69,71,72,126-129} SSc-ILD ^{100,130} SSc-ILD ¹⁰⁸ RA-ILD ^{64,131} SSc-ILD ⁷⁸ CTD-ILD, HP ¹¹⁸ iNSIP ⁷⁸ RA-ILD Sarcoidosis ⁴²

CA = cancer antigen; CX3CL1 = fractalkine; ICAM-1 = intercellular adhesion molecule 1; IFN-γ = interferon gamma; LOXL2 = lysyl oxidase-like 2; VCAM-1 = vascular cell adhesion molecule 1; YKL-40 = chitinase-3-like protein 1. See Table 1 and 2 legends for expansion of other abbreviations.

^aFor chronic fibrosing ILDs with a progressive phenotype.

with decreases in DLCO and FVC^{86,88,89}; elevated serum levels may be useful predictors of survival.^{86,90}

The potential for markers of ECM turnover to serve as biomarkers was investigated in the Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE) study.¹³² Six collagen-derived neoepitopes showed increased serum levels in patients with IPF compared with those in healthy volunteers and higher levels in patients with progressive vs stable disease. Patients with IPF showing faster rates of increase in these biomarkers over 3 months showed more rapid disease progression and reduced survival. Serum LOXL2 levels are also increased and linked to an increased risk of disease progression (hazard ratio, 5.41; 95% CI, 1.65-

17.73).^{19,27} However, a recent therapeutic trial of an anti-LOXL2-targeted therapy (simtuzumab) failed to demonstrate an effect on IPF progression.¹³³

There is some evidence that CCL18 levels can be predictive of disease progression (eg, reduction in FVC) and mortality in IPF.^{89,98} Furthermore, serum IL-6 levels have been shown to be predictive of DLCO decline.¹⁰⁰ Cancer antigen (CA) 19-9 and CA-125, which are markers of epithelial damage and secreted from the metaplastic epithelium, are also higher in patients with a progressive disease. Rising concentrations of CA-125 over 3 months were shown to be associated with increased risk of mortality.⁹⁶ The hexameric ECM glycoprotein tenascin C, expressed during tissue injury,

was shown to be highly upregulated in fibrotic lungs compared with that in normal lung tissue.⁹⁷ A correlation between lung levels of tenascin C and the progression of lung fibrosis (percentage of FVC decline over 6 months) has been demonstrated.⁹⁷ The same study reports tenascin C presence within fibroblastic foci, which represent active sites of altered wound healing in usual interstitial pneumonia.⁹⁷

Genetic variants of *MUC5B* and *TOLLIP* have been shown to have prognostic significance in IPF.^{92,93} Shortened telomere length has been associated with decreased survival.⁹⁴ Additionally, patients with IPF with autoantibodies against heat shock protein 70 in their plasma appear to have increased risk of lung function deterioration and mortality.¹³⁴

Combined assessment of multiple biomarkers appears promising on the basis that it may enable the detection of multiple aspects of disease progression (eg, epithelial cell injury and repair, alveolar macrophage activation, neutrophil recruitment or activation, or oxidative stress in the lung).⁹³ Investigation of the relationship between gene expression in peripheral blood mononuclear cells and survival among patients with IPF highlighted that survival was lower in patients with decreased expression of *CD28*, *ICOS*, *LCK*, and *ITK*.¹³⁵ These four genes were later confirmed, as part of a 52-gene expression signature, to be predictive of prognosis in patients with IPF.¹³⁶ Before that, a panel of five plasma biomarkers—MMP-7, calcium-binding protein (S100A12), IL-8, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1—also showed potential as predictors of IPF prognosis.⁸⁷

Data in Other Chronic Fibrosing ILDs With a Progressive Phenotype: At different cutoffs, serum KL-6 has been shown to predict lung function decline and/or outcome in patients with CTD-ILDs, iNSIP, and HP.^{56,57,101-103} In SSc-ILD, for example, serum KL-6 levels have been associated with the degree of inflammation and fibrosis, current impairment, and future decline in lung function (FVC or DLCO).^{80,104-108} In iNSIP, patients with higher concentrations of both KL-6 and S100A9 BALF levels had more advanced disease with notably lower FVC, DLCO, and 6-min walk test distance.⁷⁴ High serum levels of SP-A and/or SP-D have been associated with decreases in DLCO and FVC in patients with diseases such as HP, iNSIP, and SSc-ILD.^{13,38,57,113}

In patients with SSc-ILD, serum YKL-40 elevations appear to correlate with airway obstruction, low DLCO, and mortality.¹¹⁴ Among patients with HP, increases in

serum YKL-40 have been correlated with DLCO and appear to predict poor prognosis.¹¹⁵ In addition, there is some evidence that serum YKL-40 may be associated with disease activity and ongoing fibrosis in patients with pulmonary sarcoidosis and polymyositis- and dermatomyositis-associated ILD.^{116,117} High MMP-7 levels in HP have been linked with reduced survival.¹¹⁸ In patients with SSc-ILD, increased serum levels of MMP-12 appear to correlate with decreased FVC, and raised levels of tissue inhibitor of MMP-1 are associated with decreased DLCO.¹¹⁹ High levels of circulating fibrocytes have been associated with reduced lung function and an increased risk of ILD-associated mortality.⁸¹

Serum levels of CC16 correlate inversely with both lung function and disease activity in patients with SSc-ILD.⁶⁰ A randomized, placebo-controlled trial showed that IL-6 plays a mechanistic role in SSc-ILD, as anti-IL-6 treatment slowed the decline in FVC more than did placebo.¹³⁰ A large cohort study of patients with SSc-ILD reported that IL-6 predicted declines in DLCO and FVC, as well as death.¹⁰⁰

There have been numerous reports that other biomarkers of immune dysregulation may be particularly associated with SSc-ILD. Elevated levels of CRP have been linked with increased risk of progression.¹⁰⁸ Several studies have demonstrated CCL18 potential as a biomarker of change in total lung capacity, disease progression, and prognosis, although the data on its ability to predict physiologic change are mixed.^{108,126,127} BALF concentrations of CCL2 have also been associated with lung function parameters and CT fibrosis scores in patients with SSc-ILD.⁶⁹ In line with the data in IPF, patients with CTD-ILD or SSc-ILD appear to have an increased risk of mortality if they also have high levels of CA 19-9 or CA-125.^{118,123-125} Similarly, in CTD-ILD and HP, mortality risk has been shown to be raised in patients with high plasma concentrations of vascular cell adhesion molecule 1.¹¹⁸

Analysis of specimens from a multicenter study suggested that the pro-angiogenic and profibrotic factor, CXCL4, may be a useful serum biomarker for SSc-ILD because of its correlation with pulmonary fibrosis and disease severity.¹³⁷ It may also be useful for monitoring response to immunosuppressive therapy.¹³⁸ Serum CXCL10 may be useful in identifying progressive disease in SSc-ILD and in predicting outcomes in pulmonary sarcoidosis.^{42,139} SSc-ILD disease progression was also correlated with high serum levels of another chemokine,

fractalkine (CX3CL1).¹⁴⁰ High levels of CXCL13 have been associated with decreased survival in patients with CTD-ILD or HP.¹¹⁸

Telomere shortening also correlates with worse outcome in chronic fibrosing ILDs with a progressive phenotype.¹⁴¹ Candidate biomarkers for predicting the development of ILD in patients with RA include anti-citrullinated protein antibodies.¹⁴² Levels of interferon γ in the BALF of patients with RA-ILD have also been suggested to predict the risk of disease progression.⁶⁴

Patients with iNSIP with anti-MX1 autoantibodies have been reported to have improved prognosis compared with those without anti-MX1 autoantibodies.⁷⁸ In SSc, patients testing positive for anti-topoisomerase I (anti-Scl-70) antibodies and negative for anticentromere antibodies appear to have higher risk of progressive ILD.¹⁰⁸ Conversely, the presence of autoantibodies against heat shock protein 70 in patients with ILDs other than IPF does not seem to have any clinical significance.¹³⁴

There may be potential for using biomarkers to predict acute exacerbations of fibrosing lung disease with a progressive phenotype. Blood or serum levels of KL-6 or α -defensins appear promising in this regard, while levels of SP-D and leptin have also been reported to increase in patients with acute exacerbations.^{10,143} However, relatively little research has been performed in this area, and further data are required.

Therapeutic Biomarkers

Data in IPF: KL-6 is a well-established biomarker in IPF (Table 4).^{8,46,92,144-146} However, its usefulness as a potential therapeutic biomarker is complicated by conflicting data regarding the extent to which KL-6 levels are affected by antifibrotic (nintedanib or pirfenidone) treatment.¹⁴⁴

TABLE 4] Therapeutic Biomarkers

Disease	Mechanistic Pathway	Biomarker
IPF	Epithelial cell dysfunction and ECM remodeling	KL-6 ^{144,145} IGFBP-2 ⁴⁶ CRPM-1, CRPM-8, C3M, C1M ^{8,92} 5mC, mH2A1.1 ¹⁴⁶ <i>TOLLIP</i> ⁹² <i>MUC5B</i> ⁹²

5mC = 5-methylcytosine; C1M = collagen 1 degraded by MMP-2/9/13; C3M = collagen 3 degraded by MMP-9; CRPM-1/8 = CRP degraded by MMP-1/8;. See Table 1 and 2 legends for expansion of other abbreviations.

Serum concentrations of IGFBP-2 in patients with IPF were reported to be higher than those in healthy subjects.⁴⁶ The same study showed that IGFBP-2 levels in patients receiving antifibrotic therapy were significantly lower than those in untreated patients, while remaining significantly higher than those in healthy subjects.⁴⁶ The same team later reported that levels of the cell-free nucleosomes 5-methylcytosine and mH2A1.1 (epigenetic biomarkers) were significantly lowered ($P < .05$ and $P < .01$, respectively) in serum samples from untreated patients with IPF compared with those in patients treated with antifibrotic therapy (pirfenidone or nintedanib).¹⁴⁶

The rs3750920 polymorphism of *TOLLIP* seems to influence the response of patients with IPF to N-acetylcysteine therapy. Evidence for potential interaction between N-acetylcysteine and rs35705950 within *MUC5B* was also observed but not significant.⁹²

In the INMARK trial (NCT02788474) in patients with IPF, treatment with nintedanib vs placebo for 12 weeks did not affect the rate of change in C-reactive protein degraded by MMP-1 and MMP-8, suggesting that it is not a marker of response to nintedanib in patients with IPF.⁸

New Directions for Biomarker Development in Chronic Fibrosing ILDs With a Progressive Phenotype

Although numerous associations have been reported between fibrosing ILDs and serum, BALF, and genetic biomarkers, very few are purposefully or routinely used in patient clinical evaluation. Most of these potential biomarkers were investigated in an observational and retrospective manner and, more importantly, without robust validation of assays or replication of findings in separate prospective cohorts. Lack of progress towards using biomarkers in clinical practice is frustrating given that substantial numbers of studies have been published, for example, on MMP-7 and KL-6. Strategies for moving the field forward in fibrosing lung disease are outlined in Table 5. Potential biomarkers need to be thoroughly validated according to standardized guidelines. Only then will biomarkers gain regulatory approval and insurance coverage, enabling their transition from reported associations to clinical implementation. Biomarkers have the potential to help enable differential diagnosis (eg, between IPF and non-IPF fibrosing ILDs), more effective patient stratification (eg, determine the subtype of ILD or identify patients at risk of

progression), and better up-front selection of therapy. Most importantly, they may allow physicians to monitor early treatment response, which remains a huge unmet need.

Ongoing placebo-controlled clinical trials in patients with non-IPF fibrosing ILDs involve measurement of potential biomarkers as clinical end points. Chronic fibrosing ILDs with a progressive phenotype are an important emerging target for antifibrotic therapies, with two recently published clinical trials^{6,7} for patients without IPF supporting the use of antifibrotic therapy in this patient group¹⁰⁰ and the Food and Drug Administration granting nintedanib breakthrough therapy designation in this setting.

In addition to assessing biomarkers in clinical studies, we emphasize the need to continue observing biomarkers in large cohorts of patients, such as the European Scleroderma Trials and Research (EUSTAR)

group.¹⁴⁷ We also believe that it is important to continue assessing biomarkers in large registries of patients, such as the EUSTAR group or in prospective cohort studies such as the Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE) study and those in the IPF-Prospective Outcomes (IPF-PRO) registry.^{148,149}

In light of the emerging new concept of chronic fibrosing ILD with a progressive phenotype,⁶ effective treatment development will require the implementation of new specific and sensitive therapeutic biomarkers. In terms of future research directions, we believe there is the potential for combinations of blood biomarkers, or even combinations of blood biomarkers with demographic, clinical, or imaging findings, to optimize diagnosis and disease management. Thought will need to be given to avoid complexity and to ensure that combinatorial biomarker signatures retain applicability in a routine clinical setting. Multiplex biomarker assays are in development¹⁵⁰; such platforms should contribute

TABLE 5] Proposed Approaches to Biomarker Development

Research Method	Rationale
Validation of potential biomarkers according to FDA (United States), EMA (Europe), and PMDA (Japan) guidelines	Validation includes confirmation of biomarker behavior in multiple prospective cohorts. The handling characteristics of specific assays need to be defined and shown to conform to regulatory expectations to ensure clinic readiness.
Clinical trial validation of putative therapeutic biomarkers	Therapeutic biomarkers need to be tested in appropriately designed randomized controlled trials. The relevance of change in biomarker levels needs to be assessed against current end points (FVC, mortality) and in exploratory responder analyses.
Assessment of biomarkers in large registries and prospective cohort studies	Results of these studies will complement those from clinical trials and provide further support for the clinical relevance of the biomarker. Such studies may also be beneficial in determining interactions based on genotype and in identifying specific disease endotypes.
Investigation of combinations of biomarkers	Machine learning and artificial intelligence approaches afford the opportunity for identifying combinatorial biomarker signatures that may be more informative than single markers alone. Such strategies have the potential to integrate information from multiple pathogenic pathways (eg, epithelial turnover, matrix synthesis and degradation, and inflammatory cell activation). A danger of this approach is increased complexity; ultimately, any multibiomarker signature needs to retain clinical relevance to ensure use in practice.
Omics analysis of multiple biomarkers and clinical data, including pulmonary function tests, radiologic data, and disease behavior	In relation to a progressive phenotype, omics analysis may help identify the best biomarkers or combinations of biomarkers for diagnosis, treatment, and prognosis.
Unbiased biomarker discovery	The advent of novel, unbiased broad-scale proteomic assays affords the opportunity for identifying novel disease biomarkers. Any proteins identified in this way will need robust validation as outlined in the earlier steps.

EMA = European Medicines Agency; FDA = Food and Drug Administration; PMDA = Pharmaceuticals and Medical Devices Agency.

to enhanced screening, prognostication, and care. Analysis of biomarkers relating to a particular therapy could be useful for predicting the likelihood of a response to the treatment.¹⁵¹ Finally, emergence of novel categories of biomarkers (eg, exosomes, mitochondrial DNA, microRNA, quantitative imaging, transcriptomics, microbiome related) offer new and thriving areas of research that should complement and strengthen existing biomarker strategies.¹⁵²

Conclusions

In the challenging field of chronic fibrosing ILDs with a progressive phenotype, successful biomarker development should improve the diagnosis and prediction of longitudinal disease behavior (eg, identify the subgroups of patients most at high risk of disease progression), as well as monitoring and enabling measurement of the outcomes of treatment. In the future, it is hoped that the ongoing implementation of multiple biomarker analyses in large international, prospective, and adequately powered clinical studies will deliver clinically significant data that will convince physicians of the value of using biomarkers at multiple stages of the diagnosis and management of chronic fibrosing ILDs with a progressive phenotype.

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